

IMMUNOLOGIC REACTIVITY AND ALCOHOLIC LIVER DISEASE*

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IMMUNOLOGIC hyperactivity appears to play a major role in the pathogenesis and sequelae of viral hepatitis,¹ chronic active hepatitis,² granulomatous hepatitis due to schistosomiasis,³ primary biliary cirrhosis,⁴ and drug-induced hepatitis.⁵ It is assumed that the etiologic agent in these conditions evokes changes in humoral or cell-mediated immunity; it is possible, however, that a liver damaged by such agents may become antigenic. The occasional spontaneous transformation of alcoholic hepatitis into cirrhosis, despite withdrawal of alcoholic beverages and provision of a nutritious diet, is compatible with the postulate that an autoimmune process contributes to chronicity in this form of liver disease. Since this occurs in the absence of factors which initiated liver injury, this form of hepatic disease provides the clinician with a unique opportunity to test the hypothesis that damaged liver may serve as a neoantigen and perpetuate hepatic injury. This paper reviews background information and the results of investigations conducted in our laboratory which suggest that changes in immunologic reactivity are important in the development of alcoholic hepatitis and cirrhosis.

BACKGROUND

Bursal-dependent (B) cell hyperactivity with production of non-specific or specific antibodies and thymus-dependent (T) cell hyperactivity with release of biologically active factors occur concomitantly in many disease states. Microscopic lesions of alcoholic hepatitis, the precursor of cirrhosis, simulate both the Arthus phenomenon, characteristic of antigen-antibody reaction, and the host-graft type of rejection reaction seen in cell-mediated immunity. The number of B cells, as

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QUANTIFICATION OF T AND B CELLS IN ALCOHOLIC DISEASES
OF THE LIVER

<i>Histology</i>	<i>No.</i>	<i>T cells (%)</i>	<i>B cells (%)</i>
Normal	7	55 ± 2	19 ± 2
Steatosis	10	41 ± 2	20 ± 1
Hepatitis	14	34 ± 1	25 ± 3
Cirrhosis	8	36 ± 3	34 ± 11

evaluated by the labeling of immunoglobulin, is normal in the liver disease of the alcoholic, whereas a significant reduction in the number of T cells, as assessed by rosetting, is characteristic of patients with alcoholic fatty liver, alcoholic hepatitis, and alcoholic cirrhosis (Table I). The number of T cells returns to a normal range with improvement in hepatic reserve following withdrawal of ethanol and correction of nutritional deficits.⁶

Detailed studies of serum immunoglobulin patterns in patients with alcoholic hepatitis and cirrhosis reveal a nonspecific increase in immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM). Unlike chronic active hepatitis and biliary cirrhosis, there is a lack of nonorgan specific antibodies in most patients with alcoholic hepatitis or cirrhosis. Immunofluorescence techniques show that IgA separated and purified from cirrhotic sera binds to hyaline in alcoholic hepatitis; this is interpreted as evidence that autoantibodies may contribute to tissue injury in the alcoholic.⁷ Antibody-like activity against altered albumin has also been demonstrated in cirrhotic sera.⁸ It has been postulated that these antibodies which occur in the IgA class arise from immunocytes located in the liver, and that alcohol is responsible for the production of altered albumin, which may then act as an immunizing agent.

Changes have been recorded in skin tests, suggesting a change in cell-mediated immunity. However, it is difficult to interpret these findings in view of the multiplicity of exogenous and endogenous factors in the alcoholic which influence the results. Subjects with alcoholic hepatitis regularly show a significant decrease in lymphocytic response to phytohemagglutinin (PHA) and other mitogens.⁹ In contrast, an increase in migration-inhibition factor has been found after the addition of autologous liver or a purified extract of alcoholic hyaline despite the presence of a reduced index of stimulation.¹⁰



Fig. 1. Influence of phytohemagglutinin on transformation of lymphocytes from healthy nonalcoholics (controls) and alcoholics with liver disease.

NUTRITIONAL DEFICIENCY AND IMMUNOLOGIC REACTIVITY

The high incidence of nutritional deficiency in the alcoholic may explain, in part, the marked variation in the immune response encountered. Deficiencies of vitamin B₆,¹¹ folic acid,¹² and zinc¹³ have all been demonstrated to interfere with humoral and cellular immunity.

Depletion of vitamin B₆ is present in 40 to 50% of chronic alcoholics with active hepatic disease and leads to alterations in the metabolism of both protein and nucleic acid; it also depresses immune responses. Marked impairment in the response of circulating antibody to antigen stimuli has been documented in pyridoxine deficiency; there is a qualitative difference in the type of antibody formed as well as a diminution in the total quantity produced. Pyridoxine deficiency also depresses delayed hypersensitivity, leading to the suggestion that vitamin B₆ or its

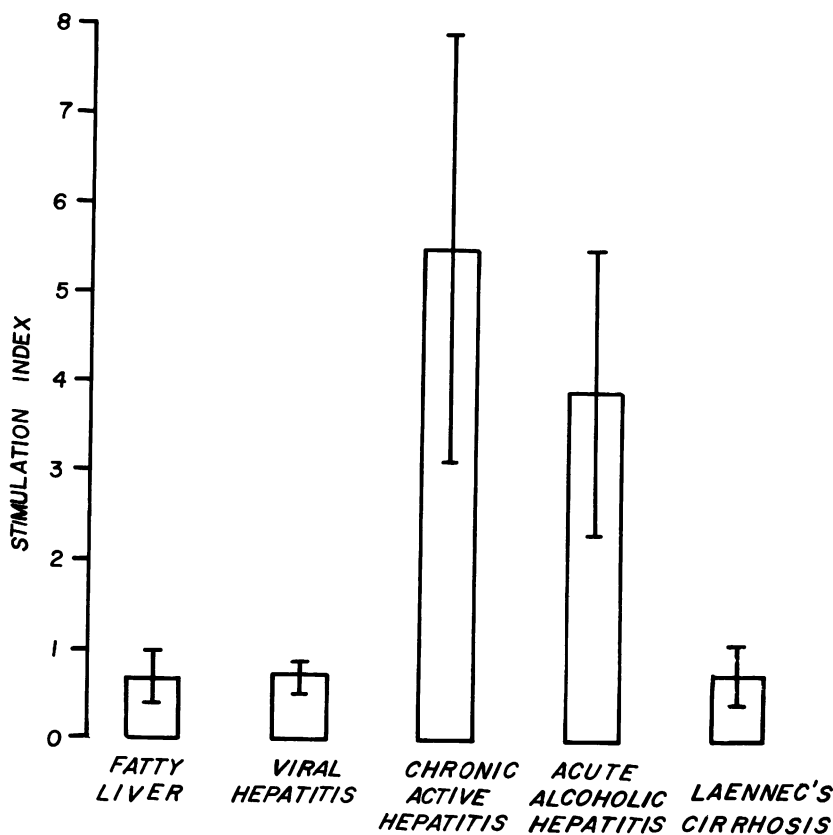


Fig. 2. Influence of ethanol or acetaldehyde on transformation of lymphocytes from alcoholics with liver disease.

coenzyme is an essential component in the sequence of reactions between sensitized cells and allergin-producing pathologic sequelae. These effects are not due to inanition, since pyridoxine antagonists inhibit antibody formation specifically and depress delayed hypersensitivity reactivity.¹¹

Folic acid deficiency, the commonest of the vitamin deficiencies encountered in alcoholics, has similar untoward qualitative and quantitative effects on the immune response.¹² Patients with megaloblastosis and reduced *in vitro* hepatic synthesis of deoxyribonucleic acid (DNA) regularly exhibit a significant reduction in lymphocyte response to PHA and other mitogens (Figure 1). An over-all decrease in leukocytes, cir-

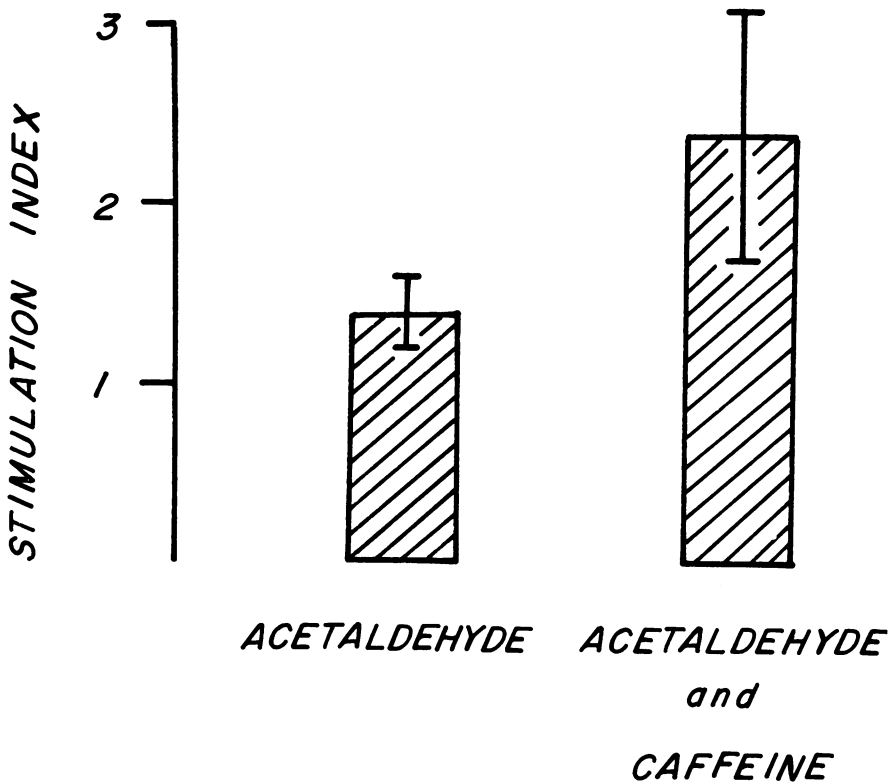


Fig. 3. Effect on lymphocyte stimulation of addition of caffeine, a phosphodiesterase inhibitor, to acetaldehyde.

culating antibodies, and reactivity to antigens is characteristic of the folate-depleted alcoholic.

Zinc, whose deficiency in the alcoholic is related both to decreased intake and to excessive urinary loss, also interferes with the metabolism of protein and nucleic acid.¹⁴ Zinc-depleted patients exhibit abnormalities in immunologic response similar to those seen in alcoholics with marked folic-acid deficiency.

ETHANOL AND IMMUNOLOGIC REACTIVITY

Ethanol, acetaldehyde, ethyl acetate, and other congeners in alcoholic beverages may cause a significant reduction in lymphocyte response. Thus, addition of more than 8 mg. of ethanol to cultured

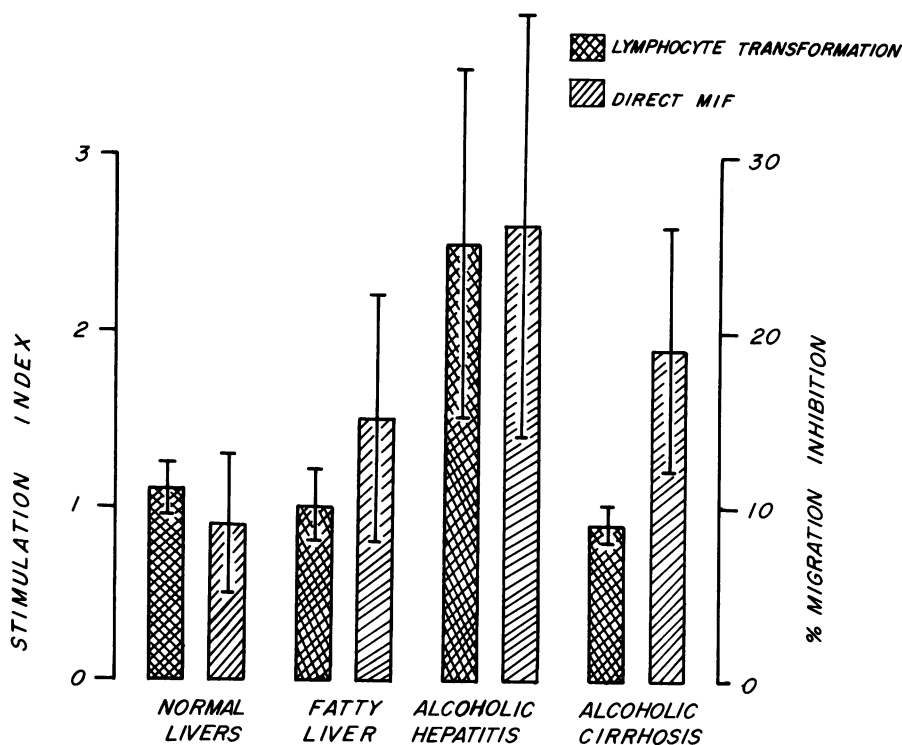


Fig. 4. Influence of autologous liver on lymphocyte transformation and production of MIF in normal subjects and in patients with alcoholic liver disease.

lymphocytes regularly diminishes responsiveness to PHA.⁹ In contrast, small, nontoxic amounts of ethanol (4 mg. or less), or acetaldehyde (10 μ g. or less), evoke a significant increase in the stimulation index of lymphocytes from patients with either alcoholic hepatitis or chronic active hepatitis (Figure 2). At the dosage level used, the failure of ethanol and acetaldehyde to evoke a similar reaction in lymphocytes from patients with fatty liver, acute viral hepatitis, and inactive cirrhosis suggests the need for alcoholic hyaline or other tissue factors which sensitize lymphocytes.

The mechanism responsible for the observed lymphocytic stimulation is not known. It seems improbable that a molecule of ethanol per se is antigenic. However, protein depolymerized by acetaldehyde could serve as an antigen. This hypothesis is supported by the finding that

acetaldehyde is generated when ethanol is added to cultured lymphocytes.⁹ As expected, there is an increase in adenosine monophosphate (5-AMP) in the sensitized stimulated lymphocytes, and the addition of caffeine produces further stimulation (Figure 3). Lymphocytic hyperactivity to ethanol or acetaldehyde disappears when clinical or laboratory evidence of alcoholic hepatitis subsides. In contrast, when a combination of ethanol and autologous liver are added, lymphocytic hyperactivity recurs.

EXTRACTS OF LIVER AND IMMUNOLOGIC REACTIVITY

Autologous liver added to lymphocytes cultured from patients with alcoholic hepatitis evokes a significant increase in the stimulation index and a decrease in the migration-inhibition index (Figure 4). This does not occur in alcoholics with normal liver, fatty liver, or inactive cirrhosis.¹⁵ These findings suggest that alcoholic hyaline, characteristic of alcoholic hepatitis, may be responsible for the observed immunologic hyperactivity. Alcoholic hyaline, synthesized by ribosomes, is an actin-like, relatively insoluble protein.¹⁶ It accumulates because of increased production or decreased dispersal. Theoretically, it could stimulate sensitized cells to release lymphocyte factors which produce a delayed reaction of the hypersensitivity type, leading to hepatic necrosis and fibrosis. Alternatively, it could stimulate antibody-forming cells to release antibodies which, in turn, by reacting with antigen could be crucial in the development of morphologic abnormalities.

An extract of alcoholic hyaline has been isolated and purified by layering liver which contains it over a discontinuous sucrose gradient, centrifuging at 99,000 g., incubating with deoxycholic acid and extracting with guanidine.¹⁷ The presence of alcoholic hyaline is confirmed by study under electron microscopy, which reveals randomly oriented fibrils. Addition of this material (10 μ g. protein per culture) to cultured lymphocytes from patients with alcoholic hepatitis causes a significant increase in the production of stimulation and migration-inhibition factor (Figure 5). A further significant increase in the stimulation occurs if both purified alcoholic hyaline and acetaldehyde are added to sensitized lymphocytes. Lymphocytes obtained from patients with hyaline associated with chronic active hepatitis or cholestasis do not exhibit an increase in reactivity to alcoholic hyaline, suggesting that different forms of hyaline may be both chemically and immunologically distinct. Of

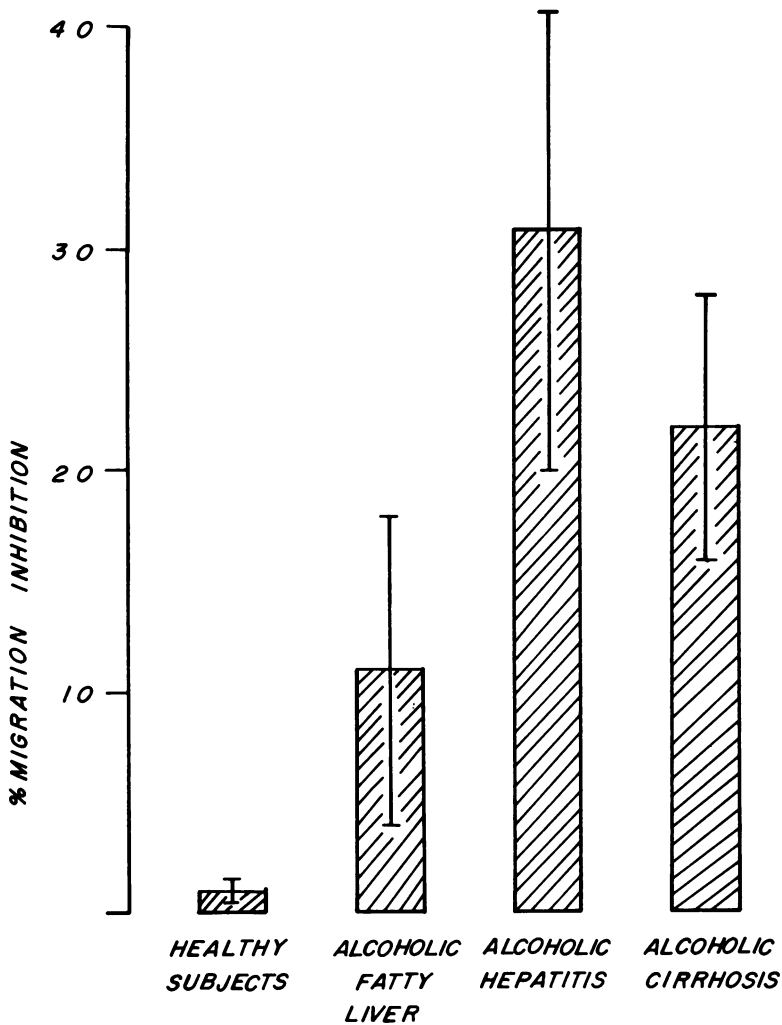


Fig. 5. Effect of a purified extract of alcoholic hyaline on production of MIF in normal subjects and patients with alcoholic liver disease.

crucial importance has been the recent demonstration in our laboratory that alcoholic hyaline induces a transfer factor.

These findings led us to investigate the possibility that immunologic hyperactivity in alcoholics may contribute to cirrhosis through release by lymphocytes of factors which increase the synthesis of collagen.

When supernatants from stimulated lymphocytes were added to cultured fibroblasts, a significant increase in the amount of radioactive proline in collagen secreted by fibroblasts was noted.¹⁸ Lymphocytes from patients with alcoholic hepatitis and a decrease in PHA responsiveness exhibited the same stimulating response to alcoholic hyaline as those with normal response to this mitogen.

DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

Responsiveness to PHA provides a reliable index to over-all lymphocyte reactivity but should be considered jointly with other parameters in evaluating the immune system of alcoholics with liver disease. In assessing results it is necessary to consider other antigens, the status of bodily nutrition, the integrity of lymphocytes, and the presence of enhancing or blocking antibodies.

Our data are compatible with the hypothesis that a damaged liver may act as a neoantigen and perpetuate hepatic injury. It would appear that alcoholic hyaline has the capacity to stimulate both T and B cells. Stimulation of T cells causes the release of a fibrogenic factor which, in combination with ethanol toxicity,¹⁹ may play an important role in the development of fibrosis and the eventual appearance of cirrhosis. Specific antibodies to alcoholic hyaline may also develop. In addition to explaining the pathogenesis of the disease, this possibility may make feasible a simple *in vitro* test to identify patients with alcoholic hepatitis. A purified preparation of alcoholic hyaline is needed for such investigations.

The concept that immunologic hyperreactivity contributes to the pathogenesis of alcoholic hepatitis and its sequelae provides many new approaches to the prevention and treatment of cirrhosis in alcoholics. Correction of deficiencies in folic acid, vitamin B₆, vitamin B₁₂, and zinc are known to increase hepatic regeneration.¹⁴ These and other nutrients will simultaneously improve antibody formation and cell-mediated hyperactivity, thereby increasing over-all immunocompetence.

Studies are currently underway in our clinic to determine the influence of corticosteroids on immunologic hyperactivity, hyaline reabsorption, and fibrogenesis.²⁰ Corticosteroids have a significant effect on the immune response, but the ultimate value of suppression of immunologic hyperactivity under these circumstances remains unknown. These drugs also decrease proline hydroxylase, which is necessary for

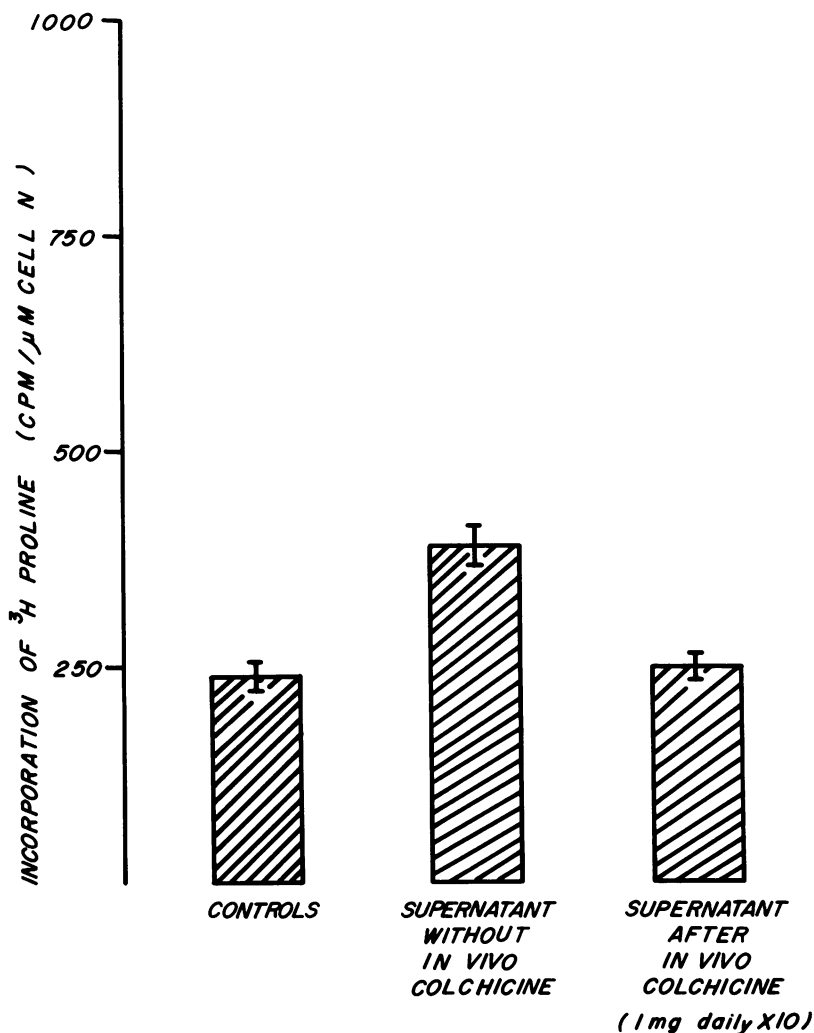


Fig. 6. Influence of colchicine on incorporation of proline into collagen by supernatants from lymphocytes stimulated with purified alcoholic hyaline.

the conversion of proline into hydroxyproline, and increase collagenase, which hastens reabsorption of collagen. Two other substances have been utilized to decrease fibrogenesis: colchicine, which inhibits the secretion of collagen,²¹ and penicillamine, which blocks the cross-linkage needed for the formation of collagen.²² In our experience, colchicine interrupts the *in vitro* fibrogenic activity of the supernatants of lympho-

cytes stimulated by alcoholic hyaline and may be a useful adjunct in interrupting progressive fibrogenesis in patients with alcoholic hepatitis (Figure 6).

SUMMARY AND CONCLUSIONS

Investigation of cell-mediated immunologic reactivity by means of lymphocyte transformation and production of migration-inhibition factor (MIF) improves the diagnostic and therapeutic perspective in the alcoholic with liver disease. Malnourished alcoholics with liver disease frequently exhibit reduced lymphocyte reactivity to the mitogen PHA; this is attributable to a reversible decrease in the synthetic capacity of DNA which occurs as a result of liver injury, nutritional deficiency, or circulating antagonists.

Immunologic hyperreactivity to alcoholic hyaline may be of crucial importance in the transformation of alcoholic hepatitis to cirrhosis. Despite a decrease in the total number of T cells and reduced responsiveness to PHA, alcoholic hyaline evokes an increase in MIF. Supernatants of sensitized lymphocytes stimulated with this material produce an increase in the incorporation of proline into collagen. This actin-like protein may serve as a neoantigen and may stimulate antibody-forming cells or T lymphocytes to release biologically active products.

Immunologic competence, as reflected in lymphocyte response to PHA, increases in the alcoholic with the improvement of hepatic reserve induced by abstinence and correction of nutritional deficiencies. Immunologic hyperactivity with release of migration-inhibition factor and fibrogenic factor persists despite progressive deterioration of the liver and eventually leads to a vicious cycle. Drugs which suppress immunologic response and reduce fibrogenesis may be valuable therapeutic adjuncts in selected alcoholics with progressive hepatic-cell destruction despite abstinence from alcohol and a good diet.

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